

Department of Health and Human Services
Public Health Services

Grant Progress Report

Review Group	Type 5	Activity PO1	Grant Number 2P01ES009581-07
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Total Project Period From: 05/07/04	Through: 10/31/08
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Requested Budget Period: From: 11/1/04	Through: 10/31/05
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1. TITLE OF PROJECT

Children's Environmental Health Center

2a. PRINCIPAL INVESTIGATOR OR PROGRAM DIRECTOR
(Name and address, street, city, state, zip code)

Frank D. Gilliland, MD, PhD
University of Southern California
Keck School of Medicine
1540 Alcazar Street, CHP 236
Los Angeles, CA 90033-9013

3. APPLICANT ORGANIZATION

(Name and address, street, city, state, zip code)
University of Southern California
2250 Alcazar Street, CSC 219
Los Angeles, CA 90033

2b. E-MAIL ADDRESS

gillilan@usc.edu

4. ENTITY IDENTIFICATION NUMBER

1951642394A1

2c. DEPARTMENT, SERVICE, LABORATORY, OR EQUIVALENT

Preventive Medicine

5. TITLE AND ADDRESS OF ADMINISTRATIVE OFFICIAL

Senior Contract & Grants Administrator
Univ. of Southern California, Dept. of Contracts & Grants
2250 Alcazar Street, CSC 219
Los Angeles, CA 90033

2d. MAJOR SUBDIVISION

Keck School of Medicine

E-MAIL: nihnga@usc.edu

6. HUMAN SUBJECTS

<input type="checkbox"/> No	6a. Research Exempt <input checked="" type="checkbox"/> Yes	6b. Human Subjects Assurance No. FWA00005906
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Exempt ("Yes" in 6a):
Exemption No.

6c. NIH-Defined Phase III
Clinical Trial ☒ No ☐ Yes

If Not Exempt ("No" in 6a):

IRB approval date 10/13/03

☐ Full IRB or
☒ Expedited Review

7. VERTEBRATE ANIMALS

☐ No
☒ Yes

7a. If "Yes," IACUC approval Date
04/28/04

7b. Animal Welfare Assurance No.
A3518-01

8. COSTS REQUESTED FOR NEXT BUDGET PERIOD

8a. DIRECT \$ 1,090,603	8b. TOTAL \$ 1,526,670
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9. INVENTIONS AND PATENTS

☒ No ☐ Yes If "Yes," ☐ Previously Reported
☐ Not Previously Reported

10. PERFORMANCE SITE(S) (Organizations and addresses)

University of Southern California
Keck School of Medicine
Department of Preventive Medicine
1540 Alcazar Street, CHP 236
Los Angeles, CA 90033-9013

11a. PRINCIPAL INVESTIGATOR
OR PROGRAM DIRECTOR (Item 2a)
Frank D. Gilliland, MD, PhD

TEL 323 442-1096
FAX 323 442-3272

11b. ADMINISTRATIVE OFFICIAL
NAME (Item 5)
Sarah J. Cusimano

TEL 323 442-2396
FAX 323 442-2835

11c. NAME AND TITLE OF OFFICIAL SIGNING FOR APPLICANT
ORGANIZATION (Item 14)

NAME Sarah J. Cusimano
TITLE Senior Contracts & Grants Administrator
TEL 323 442-2396 FAX 323 442-2835
E-MAIL cusimano@usc.edu

12. Corrections to Page 1 Face Page

13. PRINCIPAL INVESTIGATOR/PROGRAM DIRECTOR ASSURANCE: I certify that the statements herein are true, complete and accurate to the best of my knowledge. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties. I agree to accept responsibility for the scientific conduct of the project and to provide the required progress reports if a grant is awarded as a result of this application.

SIGNATURE OF PI/PD NAMED IN 2a.
(In ink. "Per" signature not acceptable.)
(b) (6)

DATE

9/8/04

APPLICANT ORGANIZATION CERTIFICATION AND ACCEPTANCE: I certify that the statements herein are true, complete and accurate to the best of my knowledge, and accept the obligation to comply with Public Health Services terms and conditions if a grant is awarded as a result of this application. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties.

SIGNATURE OF OFFICIAL NAMED IN
11c. (In ink. "Per" signature not acceptable.)
(b) (6)

DATE

9/16/04

DETAILED BUDGET FOR NEXT BUDGET PERIOD - DIRECT COSTS ONLY		FROM 11/01/04	THROUGH 10/31/05	GRANT NUMBER 5 P01 ES009581-07	
PERSONNEL (Applicant organization only)		TYPE APPT. (months)	% EFFORT ON PROJ.	DOLLAR AMOUNT REQUESTED (omit cents)	
NAME	ROLE ON PROJECT			SALARY REQUESTED	FRINGE BENEFITS
Diaz-Sanchez, David	Principal Investigator	12	30.0	(b) (6)	
Casillas, Adrian	Co-Investigator	12	10.0		
Wang, Junxiang	PGR	12	100.0		
SUBTOTALS				85,111	16,731
CONSULTANT COSTS					
Statistical consultation from the Biostat Service Core of the SCEHSC					2,781
EQUIPMENT (Itemize)					
SUPPLIES (Itemize by category)					
Plasticware, tubes, disposable pipettes, etc.					
Immunoassay materials					
Molecular assay materials					
Immunohistochemical reagents					
Cell culture reagents					19,813
TRAVEL					
Attend annual meeting					1,236
PATIENT CARE COSTS		INPATIENT			
		OUTPATIENT			
ALTERATIONS AND RENOVATIONS (Itemize by category)					
OTHER EXPENSES (Itemize by category)					
Mice					
Genotyping					
Subject Payments					7,805
SUBTOTAL DIRECT COSTS FOR NEXT BUDGET PERIOD					\$133,477
CONSORTIUM/CONTRACTUAL COSTS		DIRECT COSTS		LAREI subcontract	
				56,406	
		FACILITIES AND ADMINISTRATIVE COSTS		5,415	
TOTAL DIRECT COSTS FOR NEXT PROJECT PERIOD (Item 9a, Face Page)					\$195,298

BUDGET JUSTIFICATION**GRANT NUMBER**

5 P01 ES009581-07

Provide a detailed budget justification for those line items and amounts that represent a significant change from that previously recommended. Use continuation pages if necessary.

N/A

CURRENT BUDGET PERIODFROM
05/07/04THROUGH
10/31/04

Explain any estimated unobligated balance (including prior year carryover) that is greater than 25% of the current year's total budget.

We expect a possible carry forward in excess of 25% of the total budget, due to a 10-month delay in the year one funding of the project.

PROGRESS REPORT SUMMARY

GRANT NUMBER

5 P01 ES009581-07

PERIOD COVERED BY THIS REPORT

PRINCIPAL INVESTIGATOR OR PROGRAM DIRECTOR

Frank D. Gilliland, MD, PhD

FROM

05/07/04

THROUGH

10/31/04

APPLICANT ORGANIZATION

University of Southern California

TITLE OF PROJECT (Repeat title shown in Item 1 on first page)

Children's Environmental Health Center

A. Human Subjects (Complete Item 6 on the Face Page)

Involvement of Human Subjects



No Change Since Previous Submission



Change

B. Vertebrate Animals (Complete Item 7 on the Face Page)

Use of Vertebrate Animals



No Change Since Previous Submission



Change

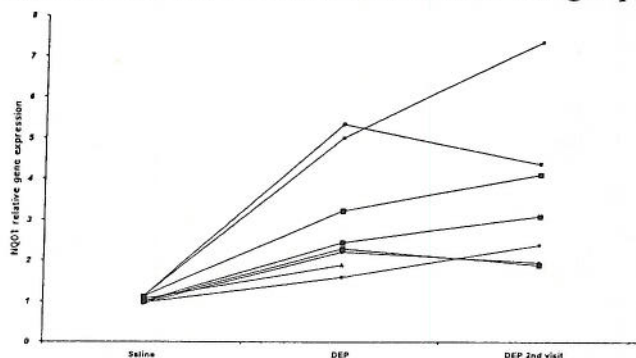
SEE PHS 2590 INSTRUCTIONS.

PROJECT NUMBER 2: POLLUTION-ENHANCED ALLERGIC INFLAMMATION AND PHASE II ENZYMES**A. SPECIFIC AIMS**

There has been no change in the specific aims of this study, they are to study the role of Phase II enzymes in regulating responses to pollutants in: children's upper airways (Aim #1); the lower airways of healthy and asthmatic individuals (Aim #2) and in mechanistic animal and cellular models of allergic inflammation (Aim #3).

B. STUDIES AND RESULTS

Aim #1: We plan to determine whether nasal challenge with DEP will induce reproducible gene expression of Phase II enzymes and compare expression in children vs. adults. We had previously shown that challenge of individuals with DEP induced GSTM1 expression in adults. In preparation for the main part of this aim, we have developed real-time quantitative PCR (RT-PCR) to measure gene expression of the other sentinel Phase II enzymes we will use in the study, NQO1 and validated its use. We identified 8 adult individuals who had functional versions of NQO1 polymorphisms and challenged them with either DEP (300 μ g) or saline during separate visits spaced 3 weeks apart. Three weeks later the subjects were recalled and challenged with DEP again. Cells were obtained from nasal lavages performed before or 24 hrs following each challenge.

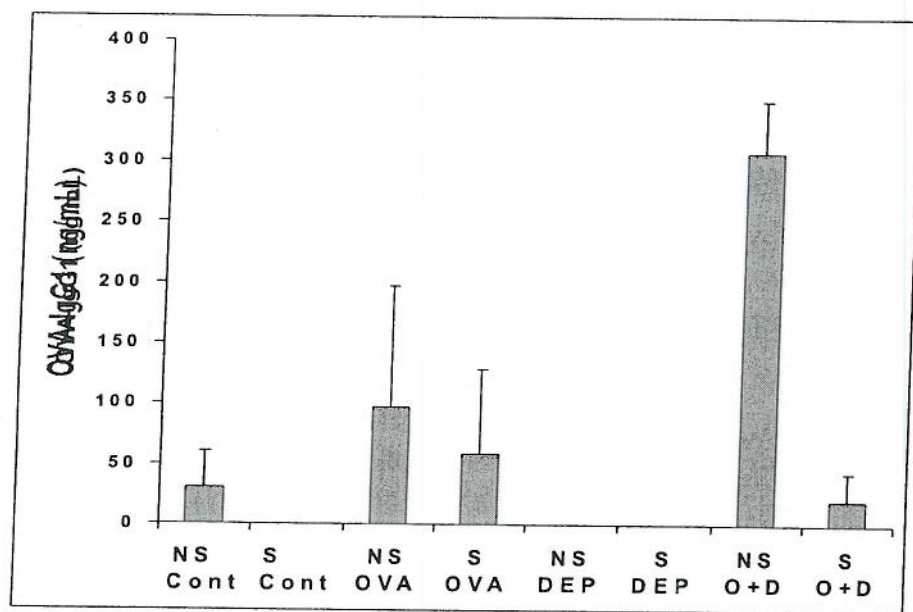


The figure shows the relative levels of NQO1 gene expression after normalization to an internal control from 8 subjects. In each case, control expression (that seen in cells from lavages after challenge with saline) of the genes were given an arbitrary figure of 1.0 and relative expression calculated with reference to baseline. In all cases, NQO1 increased but as was the case for GSTM1 gene expression, while there was considerable inter-

individual expression of NQO1, intra-individual expression was considerably less.

Aim #2: As proposed in the timeline of the proposal, we intend to commence studies on the effect of Phase II on the lower airway response to diesel in year 2. We have tested the exposure chamber and can reproducibly produce a diesel particulate exposure level which is within 10% of the target level every time. We can accurately measure particle mass, number, size and composition generated by the diesel engine.

Aim #3: We had previously shown that enhancement of Phase II enzyme expression could inhibit enhancement of IgE production by peripheral blood cells in vitro. In those experiments we used sulforaphane a potent inducer of Phase II enzymes found in cruciferous vegetables, which functions by activating Nrf2. We tested whether this same reagent could block DEP-mediated adjuvant events in vivo. Groups of six female BALB/c mice matched for age and weight (10-12 weeks) received aerosolized exposures to either: saline, ovalbumin (OVA) (1% 20 min), DEP (1 hr) or OVA followed immediately by DEP for ten consecutive days. Mice were administered either vehicle (corn oil) or sulforaphane (4.5 μ mol/mouse/day) by gavage (0.2 ml) for one week prior to the commencement of exposures up until two days after the last exposure.



The figure shows serum levels of the antigen-specific allergic antibody IgG1 14 days after allergen exposure in mice given sulforaphane (S) or no sulforaphane (NS). As expected, in the untreated group, IgG1 levels were significantly higher in mice who received both DEP and OVA than in those who were exposed to OVA alone. However, administration of sulforaphane completely ablated the adjuvant effects of DEP.

C. SIGNIFICANCE

Our results show that Phase II enzymes can be induced by our model air pollutant, DEP and are critical in regulating responses and determining susceptibility to these xenobiotics. These promising results also suggest a possible intervention strategy to augment the body's natural defense to air pollutants.

D. PLANS

In the next year we intend to recruit adults and children for Aim #1, commence recruitment for Aim #2 and repeat our mouse/sulforaphane experiment in Nrf-2 knock-out mice to confirm the action of sulforaphane is indeed through activation of Nrf-2.

E. PUBLICATIONS

None